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Original article

Correlational analyses between the production of anti-nuclear antibodies and biomarkers of acute aortic syndrome

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ABSTRACT

Objective: Markers of serological autoimmunity such as anti-nuclear antibodies (ANA) are noted in approximately 14.01% of the Chinese population. The vast majority will do not have clinical autoimmune disease. However, the possible influence of antinuclear antibodies in acute aortic syndromes (AAS) has not been elucidated.

Methods: The study group included 77 patients who underwent AAS. Serum levels of antinuclear (ANA) were tested. Patients detailed data on AAS risk factors and markers of subclinical index (including C-reactive protein, D-dimmer, Glucose, Creatine kinase) were available. Results: Of the 77 patients with AAS, 40 had classic acute aortic dissection 29 variants intramural hematoma (IMH) and 8 penetrating atherosclerotic ulcer (PAU). Among the control group, 12/76 (15.8%) were ANA positive. In marked contrast, among the study subjects 30/77 (39.0%) were ANA positive. There is a higher incidence of ANA positivity among the study group than among the control group ($p = 0.001$). The presence of ANAs was related to the occurrence of AAS ($r = 0.224$, $p = 0.005$). Meanwhile, the positive ANAs were correlated with atherosclerosis ($r = 0.167$, $p = 0.039$), the red blood cell count ($r = -0.245$, $p = 0.002$) and C-reactive protein ($r = 0.181$, $p = 0.042$).

Conclusions: Our results indicate that ANA positivity is associated with incidence of AAS, especially the incidence of intramural hematoma (IMH), suggesting that mechanisms resulting in ANA production may be involved in the development of AAS.

1. Introduction

Acute aortic syndromes (AAS) comprise classic aortic dissection, variants intramural hematoma (IMH) and penetrating atherosclerotic ulcer (PAU) (Nienaber, 2013; Goldfinger et al., 2014). Conventional risk factors such as trauma, systemic arterial hypertension, smoking, hyperlipidemia, cocaine use, and pregnancy are well-proven causes of acute aortic syndrome (AAS) (VSRJKOTSIAKA, 2009). These factors activate the the detection of individuals at risk for AAS. And several inflammatory factors have been shown to contribute to the development of AAS (Kuehl et al, 2008). The involvement of the arteriosclerosis in the AAS mechanisms remains controversial, but recent report has shown that about 25% of acute aortic syndromes have substantial atherosclerosis coexisted. So, we believe that arteriosclerosis still acts an vital role in the pathogenesis of AAS.

Antinuclear antibodies (ANA), thought of as "benign autoimmunity", may has an association with atherosclerosis (Ornella Leone et al., 2018). Some reports indicating ANA are substantially more prevalent in severe coronary atherosclerosis patients compared with in normal coronary arteries patients. Evaluation of this association may be a potentially valuable indicator of increased risk of coronary heart disease (Grainger and Bethell, 2002; Adam Mazurek et al., 2016). Solow thought ANA may involve pathways distinct from traditional risk factors and include dysregulation of endothelial cells and the immune system (Solow et al., 2018).

Although an association between autoimmunity and atherosclerosis has indicated, no relevant reports of the systemic autoimmune reaction, characterized by the presence of antinuclear antibodies (ANA) in patients with AAS have been found. Therefore,

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serum from 77 patients with contrast-enhanced CT of the aorta defined AAS in our study group was tested and compared with the serum from 76 patients with no evidence of AAS on the contrast-enhanced CT of the aorta.

2. Materials and methods

We performed a retrospective, single center observational study at the Baoding No. 1 Central Hospital, Hebei. All 178 patients admitted between November 2018 and January 2020 to the cardiovascular surgery department were included. Patients who had AAS were enrolled to the study group, and patients who had chest pain but were test to be normal through the contrast-enhanced CT (judged by two independent observers) were enrolled in the control group. The Baoding No. 1 Central Hospital institutional ethics committee approved the study. Because this is a retrospective study, and no patient's name and impact on the patient, the requirement for informed consent was waived. We excluded patients who were the presence of previously diagnosed autoimmune diseases (n = 6), concomitant treatment with immunosuppressive drugs (n = 3), any thyroid disorder (n = 4), chronic infections (n = 2), trauma (n = 5) and known malignancies (n = 5).

Ultimately, this study population included 153 patients. Baseline data such as demographics, clinical risk factors and laboratory results of these 153 subjects were obtained from the hospital's electronic medical records. AAS was diagnosed according to the published guidelines, including clinical symptoms including pretest probability of disease, laboratory data such as D-dimmer and C-reactive protein and contrast-enhanced CT (Bossone et al., 2018).

On the second morning, we collected blood samples on an empty stomach after admission, and put them into EDTA tubes, refrigerated them at 4 °C for 4 h or less, and centrifuged them. Then the plasma was removed and stored at −70 °C. Before ANA was tested, the plasma separated by centrifugation at 900g for 5 min, and by indirectly immunofluorescence on HEp-2 cells according to the manufacturer's instructions (Euroimmun AG). Titers >1:100 were regarded as positive for ANA.

3. Statistical analysis

Statistical analysis was performed using SPSS software (version 26.0 Armonk, New York). Normality of all numeric continuous variables was compared using the Mann-Whitney *U* test or *t* test. Differences in proportions were evaluated with the chi-square test. The level of statistical significance was determined at $P < 0.05$. All numerical data were presented as mean ± standard deviation, median and range, or number and percentage, as proportions.

4. Results

Of the 77 patients with AAS, 40 had classic acute aortic dissection, 29 variants intramural hematoma (IMH) and 8 penetrating atherosclerotic ulcer (PAU). None of the participants in both groups had a history of AAS. But risk factors as hypertension, smoking, hyperlipidemia and atherosclerosis were same frequent in the control group as in the study group. Only the blood pressure on admission in the study group was higher than in the control group. Patients in both groups had similar baseline and characteristics, with no differences in gender and age between the groups Table 1. The laboratory features of the groups are shown in Table 2. The white blood cell count, C-reactive protein and D-dimmer were higher in the study group than in the control group.

Among the control group, 12/76 (15.8%) were ANA positive. This is a little higher compared with previously reported for subjects (Li et al., 2019). In marked contrast, among the study subjects 30/77 (39.0%) were ANA positive. As no difference of age in the two groups, the high positive rate of ANA in the study group was unlikely to be due the influence of age. We conclude that the incidence of ANA positivity was higher in the study group than among the control group ($p = 0.001$).

Next, two specific autoantibodies anti-Ro52, anti-cANCA were performed and no difference between two groups, which suggested that neither of these autoantigens in charge of the majority of ANA positive reactions among subjects with AAS. The results are shown in Table 3. Exploratory subgroup analysis results were shown in Table 3. Among the AAD subjects, the IMH subjects and the PAU subjects, 11/40(27.5%), 16/29(55.2%) and 4/8 (50.0%) were ANA positive. We found that ANAs were positive more frequently in the IMH subjects than in the control (55.2 vs 15.8%; $P < 0.001$).

The presence of ANAs was correlated with the occurrence of AAS ($r = 0.224$, $p = 0.005$). Meanwhile, the positive ANAs were correlated with age ($r = 0.297$, $p < 0.001$) atherosclerosis ($r = 0.167$, $p = 0.039$), the red blood cell count ($r = -0.245$, $p = 0.002$) and C-reactive protein ($r = 0.181$, $p = 0.042$). The positive ANAs did not correlate with any other examined demographic or clinical variables like age, gender, smoking, glucose, D-dimer.

Table 2
The laboratory features of the groups.

	Control group	Study group	P value
Red blood cell count	4.55 ± 0.53	4.49 ± 0.67	0.571
White blood cell count	6.14 ± 2.17	9.56 ± 3.52	<0.001
C-reactive protein	2.64(1.70, 5.12)	23.50(9.16, 95.50)	<0.001
Creatine kinase	92.60(57.30, 130.70)	72.80(53.25, 131.15)	0.432
Hs-Troponin I	6.40(4.11, 9.07)	14.52(9.08, 25.45)	<0.001
D-dimmer	0.29(0.19, 0.58)	2.04(0.92, 4.57)	<0.001
Creatinine	61.20(52.43, 70.88)	76.50(63.31, 95.40)	<0.001
AST/ALT	0.97 ± 0.31	1.11 ± 0.46	0.032
Glucose	5.35(4.98, 5.75)	6.15(5.51, 7.41)	<0.001

The groups were compared using Mann-Whitney *U* test or *t* test for continuous variables,

Table 1
Characteristics and risk factors of the groups.

	Both groups	Control group	Study group	P value
Male, n (%)	113(73.9%)	51(67.1%)	62(80.5%)	0.059
Age, years	62(28, 85)	61(28, 84)	63(30, 85)	0.241
Smokers, n (%)	61(39.9%)	25(32.9%)	36(46.8%)	0.080
Hypertension, n (%)	76(49.7%)	32(42.1%)	44(57.1%)	0.063
Hyperlipidemia, n (%)	12(7.8%)	4(5.2%)	8(10.4%)	0.380
Systolic BP (mmHg)	150(130, 180)	140(125,160)	180(145, 200)	<0.001
Diastolic BP (mmHg)	90(80, 102)	85(80,95)	100(90, 110)	<0.001
Atherosclerosis,n (%)	53(34.6%)	21(27.6%)	32(41.6%)	0.070

The groups were compared using Mann-Whitney *U* test for continuous variables and χ^2 test for categorical variables.

Table 3
ANA and the other immune factors of the two groups.

	Control group	Study group			
		AAS	AAD	IMH	PAU
ANA(+), n (%)	12(15.8%)	30(39.0%)	11(27.5%)	16(55.2%)	4(50.0%)
p Value		0.001	0.133	<0.001	0.061
Ro-52(+), n (%)	6(7.9%)	9(11.7%)	5(12.5%)	3(10.3%)	1(12.5%)
p Value		0.430	0.637	0.991	1.000
cANCA(+), n (%)	3(3.9%)	5(6.5%)	1(2.5%)	4(13.8%)	0
p Value		0.731	1.000	0.170	–

The groups were compared using χ^2 test for categorical variables.

5. Discussion

In this study, defined by contrast-enhanced CT, we indicated that the positive rate of ANA in patients with Acute Aortic Syndrome was higher than that in normal subjects. Compared with the incidence of ANA positivity reported for the general Chinese population, the incidence of ANA positivity (15.8%) in our control group was a little higher (Wandstrat et al., 2006; Li et al., 2019). This phenomenon may be related to the existence of stress in patients with chest pain. And this indicated that ANA positivity is interrelated with the integrity of the aortic intima. The incidence of ANA positivity found in the study group was 39.0%. Evidence suggests that the positive rate of ANA was associated with age and C-reactive protein intead of sex in our group, where this study is inconsistent with previous research (Natorska et al., 2018; Li et al., 2019). On the one hand, the results of this study may provide a dynamic basis for the further study of the role of autoimmunity in the injury of Aortic Wall; on the other hand, it can also be tested by further investigations using a much larger cohort.

ANA, as "benign autoimmunity", was confirmed associated with all-cause mortality in the general population (Solow et al., 2015; Ferraccioli et al., 2018). In some critical reports on the role of ANA in some vessel diseases: coronary artery disease (Grainger and Bethell, 2002; Majka and Chang, 2014), venous thrombotic disease (Natorska et al., 2018) and peripheral artery disease (Kroeger and Kreuzfelder, 2004; Pertovaara et al., 2009). While patients with several vascular diseases had a positive correlation between ANA and incidence rate, there is enough evidence to support a significant effect of ANA on the pathogenesis of artery disease. Pertovaara et al. (2009) revealed that in ANA positive patients, the vascular compliance and the elasticity decreased, which fully reflected the early structural changes of arterial wall in these patients. Nonetheless, the role of ANA in AAS remains unresolved. In the study, we found that the association between ANA and AAS. The existence of this association may be because of the repeatedly implicated of a proinflammatory phenotype in vascular wall. This association may be due to recurrent proinflammatory responses in the development of vascular disease, which may lead to inflammatory dysregulation, further independently increasing acute aortic wall local injury and ANA release in circulation. In addition, ANA seroconversion may be caused by the presence of existing atherosclerosis disease, but no significant difference in the incidence of arteriosclerosis between the two groups, so the bias could be ignored.

Alternatively, our study shows that ANA is associated with c-reactive protein, which support that ANA may associated with the inflammation in the circulation. In previous studies, it was shown that the existence of ANA in blood flow may be a causative of inflammation of the blood vessel wall (Chimenti et al., 2015). And Kuehl et al., 2008 presented data that about a third of AAS patients have vascular wall inflammation, suggesting a high risk of progression to vascular disease. Solow et al. found that a low level of immune autoreactivity, as measured by ANA, may be associated with endothelial cell activity and immune up-regulation in the absence of clinically evident autoimmune disease (Solow et al., 2018). The associations described here may indicate a propensity for thrombosis mediated by dysregulated endothelial cell/platelet interactions, which could serve as a potential mechanism for further study in understanding the association of ANA with AAS. In addition, Chakravarti et al. (2015) revealed that autoimmune involved injury of large vessels, including the aorta and its branch in Large vessel vasculitides. So, characterisation of the interactions amongst ANA, inflammatory and immune mediators, and the vascular endothelium could yield novel insights into AAS pathogenesis.

In addition, we found that ANAs were positive more frequently in the IMH subjects than in the control (55.2 vs 15.8%; $P < 0.001$). This may be association with the constant stimulation of the blood vessels in the IMH. Regardless of the mechanism of this association, ANA titers may be an additional non-invasive diagnostic tool in our study population where ANA-positive antibodies, especially for nuclear antigen, correlate with AAS defined by contrast-enhanced CT.

This study has several limitations. At present, there are unresolved issues to determine whether ANA is a cause rather than a symptom, and to determine the relevance of ANA abnormalities in vital organ function and prognosis. This was a single-center retrospective and non-randomized observational study, and may have been subject to bias. Further studies should include larger populations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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